[Second Edition.]

PATENT SPECIFICATION

450,147

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Complete Specification Accepted: July 6, 1936.

COMPLETE SPECIFICATION.

Lyophilic Biologically Active Substances.

We, SHARP & DOHME, INCORPORATED, a corporation organised and existing under the laws of the State of Maryland, United States of America, of 640, North Broad Street, Philadelphia, State of Pennsylvania, United States of America, do hereby declare the nature of this invention and in what manner the same is to be performed, to be particularly described and ascertained in and by the following statement:—

This invention relates to improvements in the production, storage, preservation, restoration, dispensing and administration of biologically active substances. More particularly the invention relates to the production, storage and preservation of lyophilic biologically active substances obtained by the processes of our co-pending applications Nos. 351/35 (Serial No. 450,145) and 353/35 (Serial No. 450,146), and in the restoration, dispensing and administration thereof. The invention includes improvements both in process and in apparatus.

In the specification of our application No. 351/35 (Serial No. 450,145) we have described the production of lyophilic biologically active substances such as sera, etc., by substantially instantaneous freezing of the fresh liquid substance to the completely frozen, solid state by indirect contact with a low temperature refrigerant, removal of ice by sublimation from the solid frozen material, without previous crushing or comminution of the material, by the application of a high vacuum thereto and, if desired, with regulated warming of the material, without melting or softening of the material.

In a preferred embodiment of the said invention, the serum or other material is rapidly frozen while in a thin layer (or in successive thin layers) on the walls of a flask or container to the outside of which is applied a low temperature refrigerant such as acetone-dry ice mixture or liquid air. The container having the material rapidly frozen in a thin layer or in thin layers on its inner walls is externally exposed to the air at ordinary temperature, and is connected to a condenser maintained at a very low temperature by immersion in a low temperature refrigerant such as acetone-dry ice mixture and the apparatus is subjected to a high vacuum.

When the dyophilic biologically active substances are thus prepared, the product occupies practically the same space as the frozen material before the sublimation or vaporization of the ice therefrom. The product is porous and sponge-like with the structure of the colloidal particles apparently unchanged except for the freezing of the water and the evaporation of the ice therefrom. The product is apparently interspersed with a relatively minute net work of minute capillary pores or passages from which the frozen water has been removed by sublimation. The product is characterised by a light porous texture, is friable, and may have a density, for example, of about 0.13 in an unpulverised state. Under the microscope, pieces of the product present a capillary net-like structure.

Such lyophilic biologically active substances have improved stability and keeping properties, when properly protected, and it is an object of the present invention to supply such substances in sealed containers in which they are protected and from which they can be restored to a liquid condition, similar to the original liquid serum, etc., by the application of water thereto without destroying the vacuum in which they are maintained.

It is a further object of the invention to provide for the production of such lyophilic product from fresh liquid biologically active product in the final container in which the lyophilic product is maintained under a high vacuum until it is to be restored or used.

It is a further object of the invention to combine the preparation of the new lyophilic substance from the fresh liquid biologically active substance by carrying out the freezing operation, and the sublimation of ice under a high vacuum from the frozen solid, with the preserving of the resulting product in an evacuated container by sealing the evacuated

container after the lyophilic substance has been prepared therein, and substantially without breaking the vacuum or admitting atmospheric air.

It is a further object of the invention to provide containers for containing the new lyophilic biologically active substance which enable the lyophilic substance to be directly prepared and kept in an evacuated form and restored by the addition of water thereto without breaking the vacuum.

It is a further object of the invention to provide an attachment for attaching to bottles or other stoppered containers, which permits the production of the new lyophilic substance, the sealing of the evacuated container, and the restoration of the substance to a liquid condition by the addition of water substantially without destroying the vacuum.

This and other objects of the invention will appear from the following more detailed description.

The invention is of more or less general application to the production of lyophilic biologically active substances from fresh liquid biologically active substances such as sera of various kinds,—normal, convalescent, homologous, anti-bacterial, anti-toxin, etc., water extractives of vegetable or animal matter having biological activity which are adversely affected by time, temperature and preservatives when kept after preparation, etc. Such biologically active substances are used in the diagnosis, prevention and treatment of diseases of man and animals, and, as ordinarily prepared in a fresh liquid state, have a limited life and deteriorate in their activity or value by time, temperature, hydrolysis, oxidation, etc. As examples of such materials may be mentioned, normal horse serum, anti-toxins, such as scarlet fever, tetanus and diphtheria, anti-toxins, anti-venin and other sera, etc.

The fresh liquid biologically active substances are first converted into a lyophilic state in accordance with the process of our said application No. 35,135 (Serial No. 450,145) by placing the liquid substance in a suitable container, rapidly freezing the safe by immersing the container in a low temperature refrigerant such as liquid air, and subjecting the container to a high vacuum to remove the ice from the solid frozen material without heating it to the melting or softening point. The almost instantaneous freezing of the liquid apparently converts the water into the form of exceedingly fine ice crystals, and the high vacuum applied, with regulated warming of the container to neutralize the heat of sublimation or vaporization of the ice without melting or softening the solid frozen product results in the sublimation or evaporation of the ice crystals to leave the porous lyophilic product occupying practically the same space as the frozen solid before the removal of the ice therefrom.

According to the present invention, the freezing of the liquid sera, etc., and the sublimation or vaporization of ice therefrom are carried out in the final container in which the resulting lyophilic product is to be preserved under a high vacuum, and, after the lyophilic product has thus been prepared, the sealing of the container being effected without destroying the vacuum, whereby the lyophilic material is maintained under a high vacuum throughout the process and in the final container.

By preparing the lyophilic product in the final container in this way it retains the form or shape of the frozen material. Thus where the liquid is introduced into the container at the outset of the process and frozen in the form of a thin layer on the interior wall of the container, and when subsequent added layers of liquid are added and frozen, there will be built up a body of considerable thickness or depth in the container, and, when the ice is sublimed from the solid frozen material, the resulting lyophilic solid will occupy the same space, and retain the same shape, as the frozen material. When the material thus produced is then sealed in an evacuated container it is preserved in the form in which it is prepared, without disturbance; and, when water is later introduced through the perforable closure to restore the material to a liquid form, it will act upon this light porous body and rapidly penetrate it because the evacuated nature of the material causes the water, entering at atmospheric pressure, to be forced rapidly into the porous material.

The final containers, in which the process is carried out may vary from small containers, each containing an individual dose or unit of the material, to larger bulk containers in which a bulk quantity of the materials is prepared and stored until it is desired for use or for subdivision. The invention is applicable not only to the treatment of fresh serum or other fresh liquid biologically active material to convert it into a lyophilic form in the final container; but it is also applicable to the treatment of restored lyophilic product, where the lyophilic product is first prepared in bulb form and is subsequently restored by the addition of water and subdivided into smaller

amounts; and it is also applicable where different batches of lyophilic material are to be blended by mixing them and restoring them, or by restoring them to a liquid form before blending, to obtain a composite batch of more uniform composition. The invention is also applicable to the treatment of restored lyophilic material, restored by the addition of water, where the restored material is in a concentrated state, because of the limited amount of water added for restoring it from the lyophilic state to the liquid state and, with such concentrated liquid, the present process gives a final container with a greatly increased amount of lyophilic material as compared with the amount that could be produced in said container by the treatment of original fresh liquid material. The method of restoring the lyophilic product to a liquid product for the purpose of producing a concentrated product, or for the purpose of subdividing bulk material, or for the purpose of blending different lots of lyophilic material to form a composite batch, followed by relyophilizing of the restored liquid product is claimed in our companion application No. 353/35 (Serial No. 450,146).

The invention will be further described in connection with the accompanying drawing showing various types of containers illustrative of the invention and adapted for use in carrying out the process of the invention. In the accompanying drawings the six figures show different containers, differing somewhat from each other in respects hereinafter pointed out.

Each of the containers 1 has a neck 2 closed by a closure which is shown as a rubber stopper 3 in all the figures except Figure 7. The stopper 3 is of soft rubber, which fits tightly to maintain a vacuum and which will permit a hollow tube, such as a hollow needle to be forced therethrough, as shown at 6 in Figures 2 and 3, and the closing of the opening when the needle is withdrawn.

The containers of Figures 1, 3 and 5 have separate projecting tubes 4 through which a vacuum can be applied in carrying out the process and these tubular extensions are adapted to be sealed by heating and drawing out the tubular extensions as in the sealing of glass tubing. The tubular projections 4, when thus sealed, are shown in dotted lines.

In Figures 4, 5 and 6 a syringe plunger plug 15 forms the closure for the bottom of the receptacle and may in itself have a sufficiently tight fit to hold the vacuum in the container without moving, or an added rubber stopper 16 may be used to seal the lower end of the container and prevent breaking the vacuum until the tube is to be used. The tubular containers of Figures 4, 5 and 6 are of a size and shape adapting them for use in hypodermic syringes.

In Figures 6 there is a side opening 13 in the side of the container and a thick rubber band around that part of the container, so that a hollow needle or tube can be inserted through the thick band to evacuate the container or to reintroduce water into it. If this band is not of sufficient thickness to form a seal when the needle or hollow tube is withdrawn, the band can be turned on the container until the opening 13 is closed by an unperforated portion of the band.

In Figure 7 an ordinary bottle 1 is shown which may have an ordinary stopper or a stopper 7 held in place by an outer screw threaded cap 9. The tube 8 extends through the stopper 7 and has an upper open end 10 closed by the rubber stopper 11 and a side connection 12 serving the purpose of the tubular projections 4 of Figures 1, 3 and 5. The attachment of Figure 7 is an attachment that is applicable to ordinary bottles and enables them to be used as containers according to the present invention.

In the carrying out of the process the liquid biologically active substance, such as fresh normal serum or other fresh liquid serum or other fresh liquid biologically active product, or the concentrated liquid product or the restored liquid product, restored from the lyophilic state by the addition of water, is placed in the container 1. If these containers are unit containers which are to contain a dose or unit amount of the product such as an amount suitable for hypodermic injection or an amount of normal serum sufficient for oral administration the proper amount of liquid serum or other biologically active product is introduced into the container at the outset of the process. With bulk containers, such as flasks or other containers of several liters capacity, a large amount of fresh liquid product or of blended fresh liquid products or of restored liquid products, restored by the addition of water to the lyophilic material, or mixtures of restored liquids, is placed in the container at the outset of the process.

In each case the material in the container is subjected to rapid and almost instantaneous freezing by immersing the container in liquid air or other low temperature refrigerant as described in our application No. 351/35 (Serial No. 450,145). The container is then sub-

jected to a high vacuum, approaching a perfect vacuum, and the container is permitted to warm up by contact with the air or by regulated heating during the sublimation or vaporization of the ice from the solid frozen material and without softening or melting the material during the removal of ice therefrom, as described in our said application.

With the containers of Figure 1, 3 and 5, the vacuum can be applied for the purpose of removing the ice through the tubular projections 4 and when the ice is removed and the final lyophilic product is produced the container can be sealed by heating and melting the tubular glass projections 4 until the glass softens and closes the tube in a manner well known in sealing ampules and similar glass receptacles. The vacuum can similarly be applied through the tubular projection 12 of Figure 7 which can similarly be sealed.

With the receptacles of Figures 2 and 4 the evacuation of the container for the purpose of removing ice from the frozen material can be accomplished by forcing the tubular needle 6 or similar tube through the soft rubber stopper 3; and, when the removal of ice is complete the evacuated container can be sealed by withdrawing the tube, the rubber stopper being of a soft rubber composition which holds the vacuum when the tube is withdrawn.

In the receptacle shown in Figure 6 evacuation can be accomplished by forcing a needle or tube through the soft rubber band 14 and after the removal of ice is complete the band can be turned on the container and the tube withdrawn in a manner to leave an unperforated portion of the rubber band closing the opening 13 and holding the vacuum.

By proceeding in the manner described the liquid biologically active substance is protected from the atmosphere from the time it is inserted into the container and evacuated and the container is finally sealed in an evacuated state without having released the pressure and admitted air into the container.

With many of the biologically active substances a brief contact with atmospheric air after the liquid products have been converted into the solid lyophilic state is not objectionable. If air is admitted into the receptacle after the lyophilic product has been produced in it, the receptacle can be again evacuated, for example, by inserting a hollow needle or tube connected to a high vacuum pump through the soft rubber stopper 3 or 11, exhausting the container and then withdrawing the needle and permitting the rubber stopper to hold the vacuum in the receptacle.

The attachment of Figure 7 has the important advantage of enabling an ordinary bottle or container to be used, such as an ordinary three-ounce or five-ounce bottle. If the attachment is applied at the outset of the process the vacuum is applied through the tubular extension 12 and when the process is completed this glass projection can be heated and softened and the receptacle closed and sealed to hold the high vacuum in it.

The attachment is also a valuable attachment to use with large containers, or containers which have had the solid lyophilic product produced in it before the attachment is applied. By producing a large container having a bulk charge of the lyophilic material in it, disconnecting it from the vacuum pulp and condenser, and then applying the attachment shown in Figure 7 the container can be again evacuated and then sealed to hold the vacuum in the container.

In all cases the liquid biologically active product will be first frozen rapidly and the ice will then be removed from the solid frozen product under a high vacuum. In all cases the form in which the solid lyophilic material will remain in the final container will be the form in which the liquid material is initially frozen. Thus in Figure 1 the liquid may fill an ampule nearly full, this liquid frozen, and the ice removed from it to give a body of material 5 having the same volume and shape as the solid frozen material before the removal of ice from it. With an ampule or bottle such as shown in Figure 2 the moving of the container with the liquid material in it while it is being rapidly frozen, or the addition of the liquid material in successive amounts to build up successive layers of frozen material may give to the frozen material and to the resulting solid lyophilic material an irregular shape such as indicated at 5 in Figure 2. Similarly with the receptacles of the other figures the form of the solid lyophilic material shown at 5 will correspond to the form of the frozen liquid product before the removal of ice from it.

With all of the receptacles shown the solid lyophilic material will be kept under a high vacuum until it is to be restored for further use. This restoring is readily accomplished by inserting a hypodermic needle or other tube through the soft rubber stopper. Since the containers are under high vacuum the water will be drawn in by the vacuum and will rapidly penetrate the evacuated porous solid

material. By thus adding water to the lyophilic product in the container without breaking the vacuum, prompt and complete restoration to a liquid state is readily accomplished. If the water completely fills the evacuated container and if atmospheric pressure is applied to the water it will force the water into all parts of the porous material. If the water fills the container only partly but covers the porous material, and if atmospheric air pressure is then permitted to act on the surface of the water it immediately forces it throughout the evacuated porous material and promotes the restoration.

This action of the water when it is introduced into the evacuated container forms a valuable and important test of the vacuum in the filled container and, if the container has lost its vacuum, and the water does not readily penetrate the material when it is introduced and atmospheric pressure is admitted, it is a warning that the material has not been properly maintained under a vacuum.

In all cases where the vacuum has been maintained the restoration of the lyophilic material to a liquid state for administration or for further use is readily accomplished in the manner above described and a liquid product produced comparable in properties with the original fresh serum or other biologically active liquid product.

It is not necessary to maintain the vacuum when the product is being restored in cases where the product is to be removed from the receptacle, for example, for oral administration; and in that case, the stopper 3 or 7 can readily be removed and water introduced through the opening to dissolve or restore the lyophilic material to a liquid state.

It will thus be seen that the present invention provides an improved method of providing solid lyophilic biologically active materials in a state that insures their protection and preservation in the final container; that the removal of the ice from the solid frozen material under a high vacuum can be followed directly with the sealing of the evacuated container to hold the lyophilic material from contact with air until it is to be used; and that the material can be restored to its liquid state without destroying the vacuum and without contact of air until it is restored to its liquid condition.

When the restored liquid material is to be used in a hypodermic syringe the containers shown in Figures 4, 5 and 6 are advantageously employed with the removal of the stopper 16 where this is used, the perforation of the stopper 3 with one end of a hypodermic needle, or with a hollow tube connected to a hypodermic needle, and the use of the plunger 15 as the piston to force the restored liquid out into and through the needle for administration.

Having now particularly described and ascertained the nature of our said invention and in what manner the same is to be performed, we declare that what we claim is:—

1. A process of preparing lyophilic biologically active substances by the process described and claimed in Specification No. 351/35 (Serial No. 450,145) in a final container, which comprises introducing liquid biologically active substances into the container, substantially instantaneously freezing the same therein to a completely frozen solid condition by indirect contact with a low temperature refrigerant, removing ice by sublimation from the solid frozen material, without previous crushing or comminution of the material, by application of a high vacuum thereto without melting or softening of the material, and sealing the resulting solid lyophilic product in the container in an evacuated state, the sealing of the container being effected substantially without destroying the vacuum whereby the lyophilic material is maintained under a high vacuum throughout the process and in the final container.

2. A process according to claim 1 wherein the container is connected to high vacuum producing means by means of a glass tube, said container being sealed by heating and softening said tube until the glass softens and closes the tube.

3. A process of preparing lyophilic biologically active substances obtained according to claim 1 or 2 in the liquid state in said final container, which comprises adding water to the evacuated container without destroying the vacuum before the water is applied.

4. A container when used in carrying out the process claimed in claim 1, 2 or 3 comprising a perforable rubber closure adapted to permit the insertion of a small tubular member therethrough and to seal the container and hold the vacuum when said tubular member is removed.

5. A container when used in carrying out the process claimed in claim 1, 2 or 3, having a neck and rubber closure for said neck, said tube having an opening therethrough with a glass tube fitting said opening, said tube having at one end a perforable rubber stopper adapted to permit the insertion of a small tubular member therethrough and the withdrawal thereof and the closing of the opening made by said member, and said

glass tube also having a sealed side connection adapted to be used, before sealing, to permit the evacuation of the container.

6. The process for the production of lyophilic biologically active substances in a final container substantially as described.

Dated the 4th day of January, 1935.

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450,147 COMPLETE SPECIFICATION

2nd Edition

[This Drawing is a reproduction of the Original on a reduced scale.]

Fig.1.

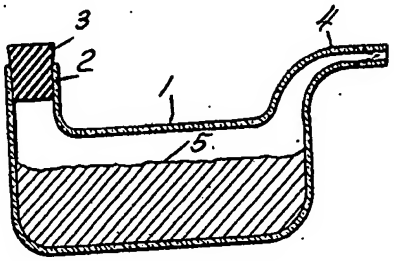


Fig.2.

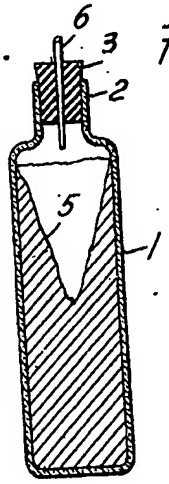


Fig.3.

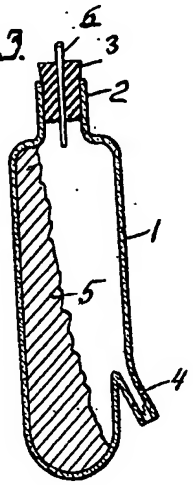


Fig.7.

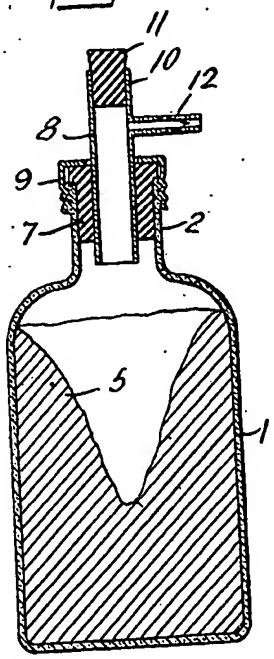


Fig.4.

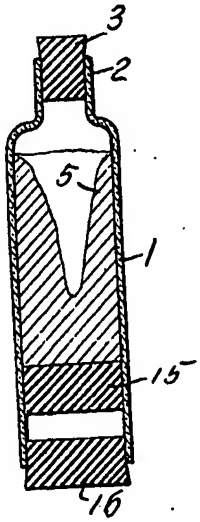


Fig.5.

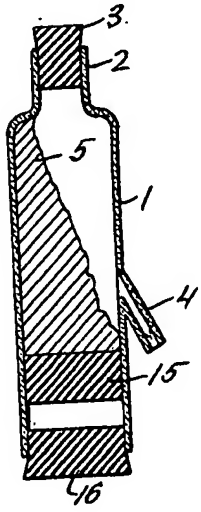
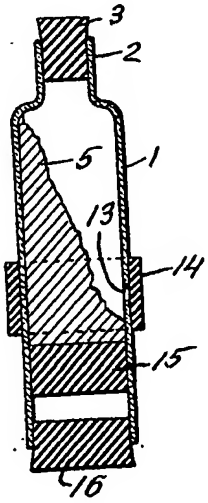


Fig.6.



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